Supplementary Information (SI) for RSC Sustainability.
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SUPPORTING INFORMATION

CO_2-Mediated Conversion of Furfuryl Alcohol to 4-Hydroxycyclopentanone and its Toxicological Assessment

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Materials and Methods

1. General Information

Furfuryl alcohol (FAL) was distilled before its use and storage under nitrogen atmosphere in a refrigerator. The others reagents and solvents were used as received.

Table S1. Name, CAS number, purity and suppliers of the reagents used

Name	CAS number	Purity / Concentration	Supplier
Furfuryl Alcohol	98-00-0	97 %	Sigma Aldrich
2-Methyltetrahydrofuran	96-47-9	≥99.5 %	Sigma Aldrich
Deuterium oxide	7789-20-0	99.9 %	Sigma Aldrich
Dimethyl sulfone	67-71-0	98 %	Sigma Aldrich
4-Hydroxy-2-cyclopentenone	61305-27-9	98 %	BLDpharm
Methanol	67-56-1	≥99.8 %	Sigma Aldrich
Menadione	58-27-5	≥98 %	Sigma Aldrich
Calicheamicin	108212-75-5	≥95 %	Pfizer

2. Materials and Methods

2.1 Catalytic Reaction Procedure

The catalytic experiments were carried out using a Parr reactor (75 mL vessel) equipped with a 4871 Process controller and an equilabo software system. In a typical procedure, 6 mmol of furfuryl alcohol (FAL), 12 mL of deuterium oxide (D_2O) and 7 mL of 2-methyltetrahydrofuran (MeTHF) were loaded into the reactor. After sealing, the reactor was flushed with CO_2 and pressured to the desired pression at ambient temperature. Then, the reactor was heated to the desired temperature with a heating ramp of 3.45 °C/min and a stirring speed fixed to 480 rpm. Once the reaction is completed, the reactor was quickly cooled down in an ice bath in order to quench the reaction and then depressurized. Blank experiments were performed following the same protocol without the injection of CO_2 gas.

2.2. Analysis and quantification of reactions products

After reaction, the recovered biphasic media (D_2O/MeTHF) was homogenised by the addition of 3.5 mL of methanol. Straightaway, aliquots were taken from the reaction media and filtered over a Millipore PES filter with a pore size of 0.45µm prior analysis by ^1H NMR. Quantitative H^1 NMR experiments were performed by mixing 10 mg of dimethyl sulfone (used here as an internal standard) and 50 mg of the crude reaction sample, dissolved in 0.4 mL of deuterated deuterium oxide. Acquisition parameters were: Number of scan (ns): 16; acquisition time (AQ): 780s and relaxation delay (D1): 40s in a Aimant Bruker Untrashield 500 mHz equip.

In order to determinate the exact amount of reactants and products obtained during the reaction, the purity (Px) of each compound was calculated by the following equation.[1]

$$P_x = \frac{I_X}{I_S} \times \frac{N_S}{N_X} \times \frac{M_X}{M_S} \times \frac{w_S}{w_C} \times P_S$$

Where I, N, M, w and P are the signal area, number of protons, molecular mass, weight mass and purity (mass fraction) respectively for the compound (X) and the internal standard (S).

The exact mass of X in the crude reaction sample (w_X) was calculated by:

$$w_X = P_X \times w_{TC}$$

To determinate the moles of X it was used the follow equation:

$$n_X = \frac{w_X}{M_X}$$

The conversion, yield and selectivity were calculated using the following equations:

$$Yield\ (\%) = \frac{(\frac{n_X}{a})}{(\frac{n_{R,0}}{b})}$$

$$Conversion\ (\%) = \frac{n_{R,0} - n_{R,t}}{n_{R,0}} \times 100$$

$$Selectivity\ (\%) = \frac{Yield\ (\%)}{Conversion\ (\%)} \times 100$$

Where $n_{R,0}$ is the initial moles of the reactant, $n_{R,t}$, the moles of the reactant after a determinate time, and a and b are que stoichiometric coefficient of X and R respectively.

Figure S1. Example of crude ^{1}H NMR spectra collected at total conversion of FOL (160°C, 20 bar CO_2, 5 min). Me-THF is the reaction solvent, methanol (MeOH) was added to dilute aliquots and dimethylsulfone was used as an internal standard for quantification.

2.3. Calculations details

All calculations were made using Gaussian 16 rev B.01, at the wB97xD/6-311++G(d,p) level of theory. This level of theory was selected because of its overall performance in modelling organic reactions[2-6] and intermolecular interactions, both from the long-range correction and inclusion of dispersion.

Solvation was taken into account using the PCM solvation model, and exploiting the default parametrisation of water. Geometries were optimised without constraints and the nature of stationary points was confirmed by frequencies calculations. Hereafter, all energies are ZPE-corrected electronic energies.

In a first series of calculations, we studied the potential implication of the acid catalysts in the cyclisation reaction of 4-oxopent-2-enal. To this end, we located the transition state associated to the cyclisation step for 4-oxopent-2-enal alone (no catalyst) and in double H-bonding interaction with HCO_3^-, HCO_3H and HCO_2H. In the absence of catalyst, the TS is found to lie at 29,8 kcal/mol above the starting reagent. In the presence of HCO_3^-, the TS is now found 18.0 kcal/mol above the pre-reacting complex, and the imaginary vibration mode is associated to both the formation of the desired C-C bond and the transfer of a H atom from the enol group to the ketone group. A comparable vibration mode is found in the case of carbonic acid (activation energy of 17.0 kcal/mol) and formic acid (18.0 kcal/mol).

In a second series, we then studied the trapping of the transient cyclic oxocarbenium ion formed by the dehydration of FOLH+ with either formate or bicarbonate anions. Optimization of these neutral adducts confirmed these are stable geometries on the PES, with relative energies with respect to the starting ions of -31.9 and -25.0 kcal/mol, respectively. In the case of HCO_3^-, we could further identify a transition state associated to a complex reaction event, composed a intermolecular proton transfert from the bicarbonate to the endocyclic furanic ring O atom, an endocyclic C-O bond cleavage, and a decarboxylation (see Scheme 2 in manuscript). This TS is found to lie at 25.5 kcal/mol above the intermediate, and thus only 0.5 kcal/mol above the isolated ions. Overall, the energy cost for this step is thus expected to be very moderate.

2.4. Cells culture and treatments

HepG2 (ATCC HB-8065) and Caco-2 (ATCC HTB-37) cells were cultured in Minimum Essential Medium α (MEM α, Gibco) or Dulbecco's Modified Eagle Medium (DMEM, Gibco), respectively, supplemented with 10 % fetal bovine serum (FBS, PAN BIOTECH) for HepG2 cells or 20 % FBS for Caco-2 cells, and 1 % antibiotics (penicillin-streptomycin, Gibco). Cells were grown at 37 °C in a humidified atmosphere containing 5 % CO2 and subcultured every 2-3 days.

All chemicals were resuspended in DMSO vehicle and serial dilutions were performed in DMSO and medium, when necessary, in order to obtain the indicated concentration with DMSO 0.1 % final in cellular assays. The DMSO 0.1 % condition was therefore used as negative control in all further assays. Calicheamicin 10 nM (Pfizer, France), a radiomimetic, was used as positive control in viability or genotoxicity assays. Menadione 25 µM (Sigma Aldrich) was used as positive control in oxidative stress assay.

2.5. Cell Viability Assay

Caco-2 and HepG2 cells were seeded in duplicate at a density of 15,000 cells per well in 96-well plates. One day after seeding, cells were treated during 24 h (timepoint R0). Cells were then incubated in fresh complete culture medium for further 24 h (timepoint R1). Viability was assessed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer's instructions. The DMSO 0.1 % condition and Calicheamicin 10 nM were used as negative and positive control respectively. Three independent experiments were performed.

2.6. Oxidative Stress Assay

Caco-2 and HepG2 cells were seeded in duplicate at a density of 15,000 cells per well in 96-well plates. One day after seeding, cells were treated during 24 h. Menadione 25 µM was used as oxidizing agent and positive control whereas DMSO 0.1 % was used as negative control. In order to assess the oxidative status, cells were incubated with CellRox Green Reagent (Invitrogen) as previously described,[7] washed in PBS and fixed with 4% paraformaldehyde. After three washes in PBS, cell nuclei were counterstained with 1 µM DAPI. The oxidized CellRox fluorescent and DAPI signals were analyzed using an ImageXpress Micro Confocal High-Content Imaging System (Molecular Devices) with a 20 X objective. The fluorescent signal intensity was quantified in at least 5.000 cells per sample (Unit Arbitrary, U.A). The signal intensity of each nucleus was quantified with

the MetaXpress software (Molecular Devices). The mean intensity of each condition was compared to the untreated condition. Three independent experiments were performed.

2.7. Genotoxicity analysis

Caco-2 and HepG2 cells were seeded in duplicate at density of 15,000 cells per well in 96-well plates. One day after seeding, cells were treated during 24 h. Calicheamicin 10 nM was used as a positive control for genotoxicity. The immunofluorescence assay was performed as previously described.[8] Briefly, cells were washed in PBS, fixed with 4 % paraformaldehyde, permeabilized with 0.5 % Triton X-100, then blocked with 3 % BSA and 0.05 % IGEPAL CA-630. Cells were stained with anti-γH2AX primary antibody (Merck/Millipore 05-636-1) for 2 h at room temperature in blocking solution, and washed three times with PBS 0.05 % IGEPAL before incubation with secondary antibody (Alexa Fluor 546 Goat anti-mouse, Invitrogen) for 1 h at room temperature. DNA was stained with 4.6-diamino-2-phenyl indole (DAPI) at 100 nM. Cells were analyzed using an ImageXpress Micro Confocal High-Content Imaging System (Molecular Devices) with a 20 X objective. The level of the nuclear γH2AX signal was quantified in at least 5,000 cells per sample (nuclear intensity U. A) with the MetaXpress software. The mean intensity of each condition and the number of nuclear foci were compared to the untreated condition. Three independent experiments were performed.

2.8. Statistical analysis

Statistical analysis of the data was performed using Prism 10 software (GraphPad). One-way ANOVA analysis of variance followed by Dunnet's *post hoc* test for the comparison of different conditions to the negative control were performed. The results are expressed as the mean ± standard error of the mean (SEM) and the statistical significance indicated (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$).

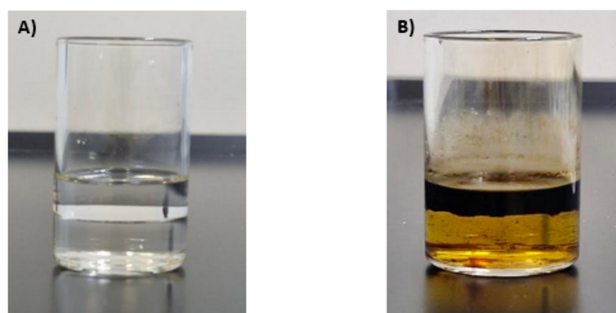

Figure S2. Color of the reaction before (A) and after (B) reaction at 200° C

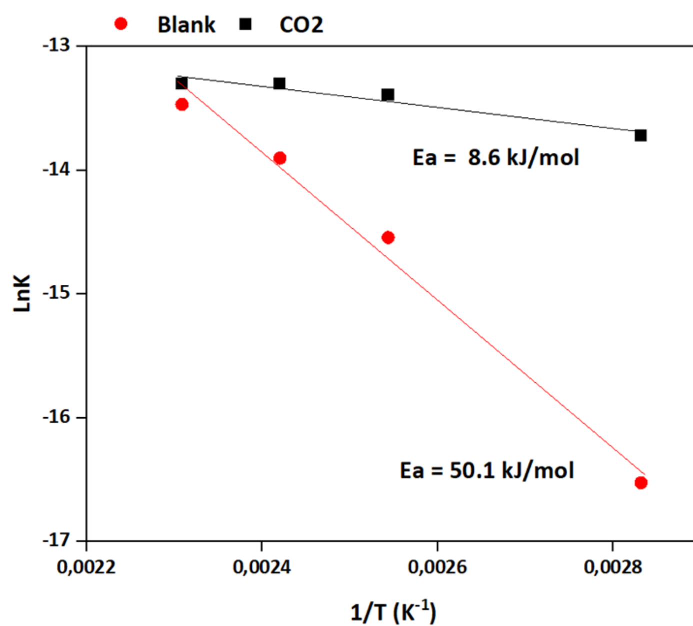

Figure S3. Arrhenius plot for the calculation of Ea in the production of 4-HCP with and without CO_2.

Table S2. Study of the temperature effect in the FAL Piancatelli rearrangement to produce 4-HCP with and without CO_2.[a]

Entry	Temperature (T^a)	CO_2 (bars)	Time (min)	Conversion (% mol)	Yield of 4-HCP (% mol)	Selectivity to 4-HCP (% mol)
1	120	-	5	-	-	-
2	120	-	60	29	20	69
3	120	20	5	38	9	24
4	120	20	60	92	52	57
5	120	20	120	100	42	42
6	140	-	5	-	-	-
7	140	-	60	55	37	67
8	140	-	120	61	57	93
9	140	20	5	63	6	10
10	140	20	60	100	51	51
11	160	-	5	19	-	-
12	160	-	60	80	44	55
13	160	-	120	100	50	50
14	160	20	5	100	55	55

[a] Reaction Conditions: 6 mmol FAL, D_2O/MeTHF (60/40), heating ramp 3.45°C/min.

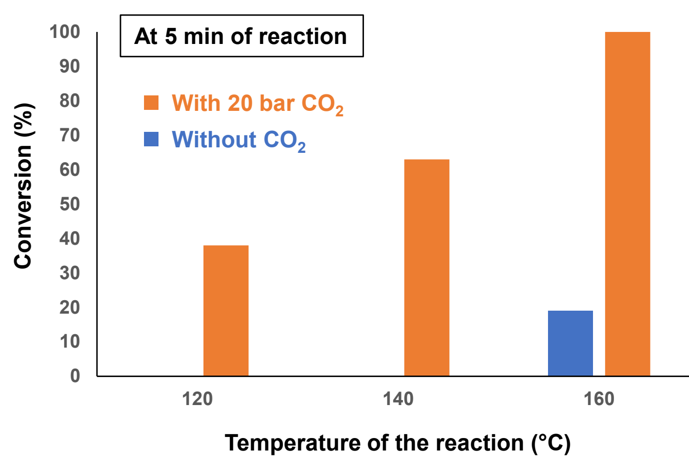

Figure S4. Conversion of FOL after 5 min of reaction at 120, 140 and 160 °C, with (orange bars) and without CO_2 (blue bars). Data extracted from Table S2.

Table S3. Study of the amount of the pressure of CO_2 in the production of 4-HCP from FAL by Piancatelli rearrangement.[a]

Entry	CO_2 (bars)	Time (h)	Conversion (% mol)	Yield of 4-HCP (% mol)	Selectivity to 4-HCP (% mol)
1	-	0.5	18	12	67
2	10		24	-	-
3	20		87	40	46
4	30		84	27	32
6	-	1	29	20	69
7	10		90	25	28
8	20		92	52	57
9	30		100	42	42
11	-	2	30	21	70
12	10		94	25	27
13	20		100	42	42
14	30		100	41	41
16	-	3	31	22	71
17	10		100	40	40
18	20		100	42	42
19	30		100	41	41
21	-	6	85	55	65
22	10		100	38	38
23	20		100	41	41
24	30		100	40	40

[a] Reaction Conditions: 6 mmol FAL, D_2O/MeTHF (60/40), 120° C, heating ramp 3.45 °C/min

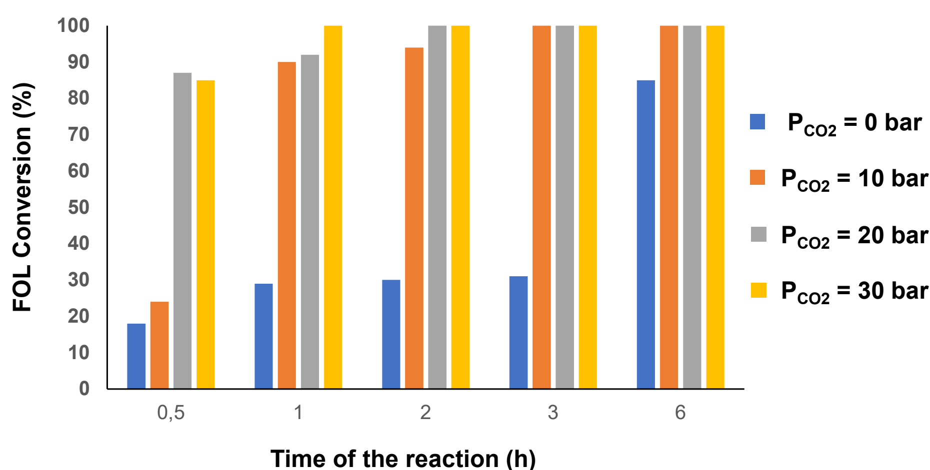

Figure S5. Conversion of FOL over time at 0 (■), 10 (■), 20 (■) and 30 (■) bars of CO_2 pressure at 120°C. Data extracted from Table S3. Note that without CO_2 pressure, an induction period is observed, which is explained by problem of homogeneity at this temperature when picking-up aliquots, in particular at low conversion. Under CO_2 pressure, this induction period does not appear mainly because of a better emulsification of the reaction media.

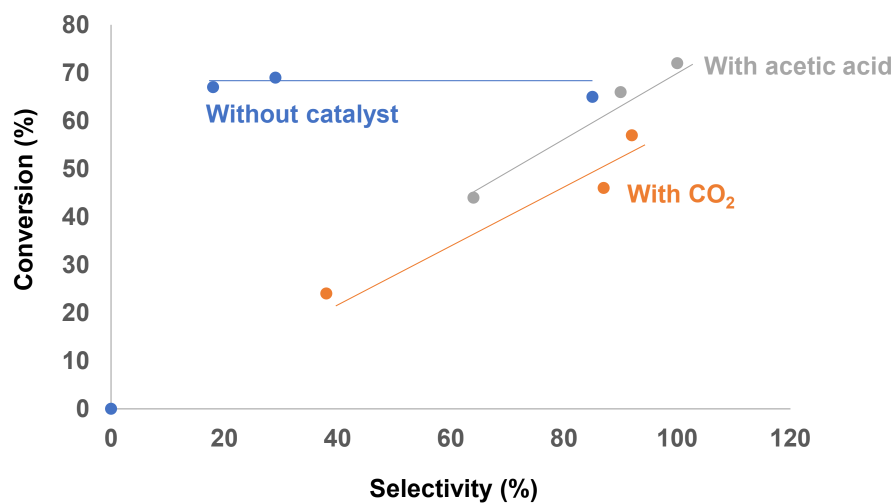

Figure S6. Plot of the FOL conversion as a function of the 4-HCP selectivity at 120°C with CO_2 (orange), with acetic acid (grey) and withot any catalyst (blue).

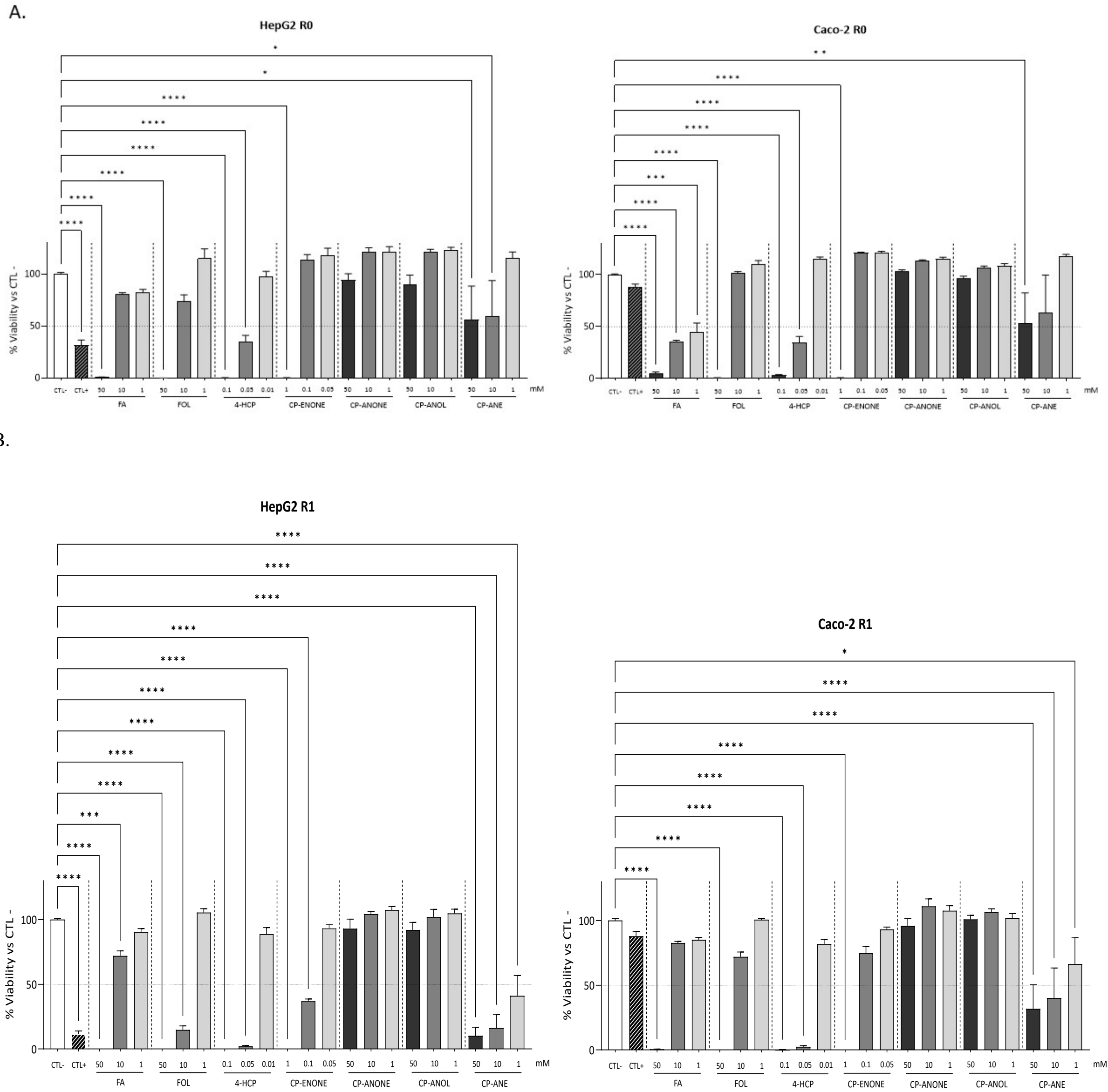

Figure S7. Viability analysis of FA, FOL and derived compounds *in vitro*. HepG2 and Caco-2 cells viability was assessed after a 24 h-treatment (R0) with the indicated compounds, at different concentrations. The viability was then compared to the vehicle-treated cells (DMSO 0.1 %) as negative control (A). HepG2 and Caco-2 cells were treated during 24 h, then incubated in fresh culture medium for further 24 h in order to assess the loss of viability at a later timepoint (R1). Viability was assessed and compared to the negative control (B). Three independent experiments were achieved. The mean ± standard error of the mean (SEM) of a representative experiment is shown, the statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001) and 50 % viability loss (horizontal line) are indicated.

Table S4. Toxicology results of different molecules investigated in Caco-2 intestinal cells and HepG2 hepatic cells.

Entry	Compound	HepG2 IC_{50} range (R1)	Caco-2-IC_{50} range (R1)
1	FA	10-50 mM	10-50 mM
2	FOL	1-10 mM	10-50 mM
3	4-HCP	10-50 µM	10-50 µM
4	CP-ENONE	0.05-0.1 mM	0.1-1 mM
5	CP-ANONE	> 50mM	> 50mM
6	CP-ANOL	> 50 mM	> 50 mM
7	CP-ANE	1 mM	1 mM

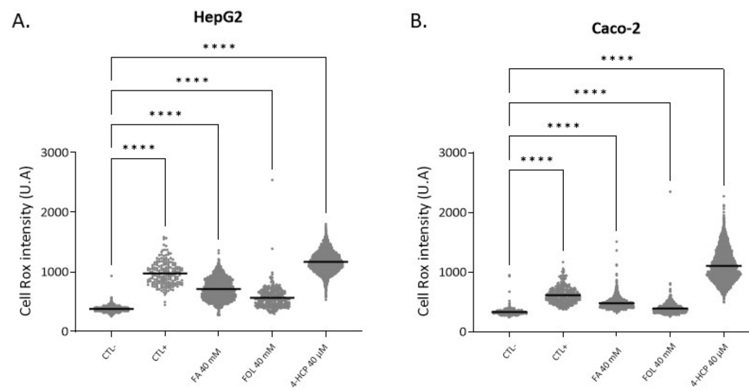

Figure S8. Oxidative stress analysis of FA, FOL and 4-HCP *in vitro*. HepG2 (A) and Caco-2 (B) cells were exposed 24 h to the indicated compounds, at different concentrations. The oxidative stress level was quantified and compared to the vehicle-treated cells (DMSO 0.1 %) as negative control. The mean ± standard error of the mean (SEM) of a representative experiment and statistical significance (****P ≤ 0.0001) are indicated.

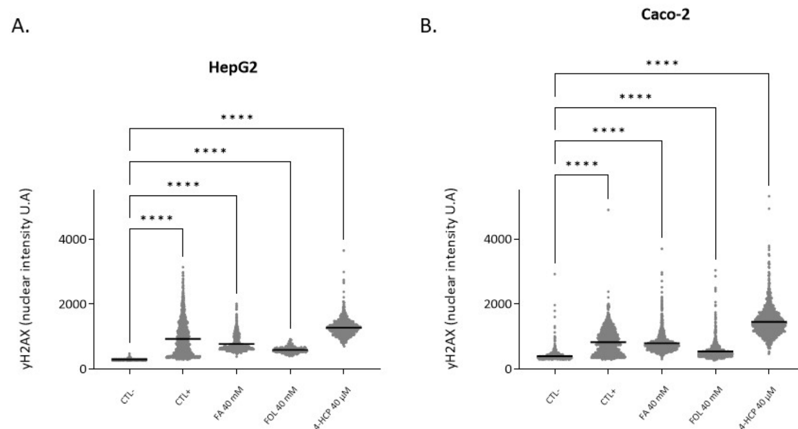

Figure S9. Genotoxicity analysis of FA, FOL and 4-HCP *in vitro.* HepG2 (A) and Caco-2 (B) cells were exposed 24 h to the indicated compounds, at different concentrations. The genotoxicity level was quantified and compared to the vehicle-treated cells (DMSO 0.1 %) as negative control. The mean ± standard error of the mean (SEM) of a representative experiment and statistical significance (****P ≤ 0.0001) are indicated.

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